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ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF *ACINETOBACTER BAUMANNII* WITH PHENOTYPIC DETECTION OF EXTENDED SPECTRUM BETA-LACTAMASES (ESBL) & METALLO BETA LACTAMASES (MBL) PRODUCTION FROM VARIOUS CLINICAL SAMPLES AT A TERTIARY CARE HOSPITAL, NORTH INDIA

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ABSTRACT

Background: *Acinetobacter baumannii* is one of the major pathogens causing nosocomial infections or healthcare-associated infection due to the emergence of resistance to various antimicrobial agents. Resistance due to antimicrobial degrading enzymes Like ESBL and MBLs is now a worldwide problem and a major reason for concern for the treating physicians.

Materials and methods: This is a cross-sectional study, a total of 55 *Acinetobacter baumannii* isolates from various clinical samples from November 2019 to April 2020.

Acinetobacter baumannii isolates from various clinical samples were screened for Cephalosporins and Meropenem resistance for the detection of ESBL and MBL production. Carbapenemases production was confirmed by DDST and EDTA DST method for ESBLs and MBLs.

Results: A total of 55 *Acinetobacter baumannii* isolates of in all clinical samples. Out of isolating Extended Spectrum Beta-Lactamases (ESBL) detection through screening method was 45 (81.82%) & confirmatory detection by DDST method was 42 (76.36%) and MBL (Metallo-β-Lactamases) detection by the screening method Carbapenems resistance were 40 (72.73%) and confirmatory detection EDTA DST method 35 (63.64%) respectively. Out of total isolates, most of the samples were Endotracheal Tube 35 (63.64%) & Blood 9 (16.36%). Detection of ESBL by phenotypic method out of 45 (81.82%) samples resistance of 3rd generation Cephalosporins were Endotracheal aspirate 31 (56.36%) & blood 6 (10.91%) and 42 (76.37%) ESBL confirmatory detection by DDST method were 30 (54.54%) Endotracheal aspirate & 5 (9.09%) Blood respectively. Out of 40 (72.73%) MBL Positive by Carbapenems resistance detection with Phenotypic Screening test Endotracheal aspirate 31 (56.36%) & Blood 5 (9.09%) and out of 35 (63.64%) Phenotypic Confirmatory by EDTA DST is Endotracheal Tube 30 (54.54%) & Blood 4 (7.27%) respectively.

Acinetobacter baumannii isolates were 100% sensitive to Polymyxin B and Colistin for, confirmed MBL & ESBL production. Resistance to 100% Ceftazidime and Ceftriaxone for ESBL & MBL producer *Acinetobacter baumannii* confirmed ESBL and Meropenem and Imipenem both are antibiotics were 100% resistance in MBL producer.

Conclusion: This study gives an alarming sign of a high ratio of multidrug resistance due to the production of extended-spectrum Beta-Lactamases and Metallo beta Lactamases, respectively.

KEYWORDS : Acinetobacter, Carbapenems, MBL, ESBL, DST, and EDTA.

INTRODUCTION

Acinetobacter baumannii is a kind of aerobic, non-motile, Gram-negative coccobacillus, non-lactose fermentative, oxidase-negative, and Catalase positive organism [1].

In the health care units, *Acinetobacter baumannii* has become a common pathogen, which can infect the endotracheal aspirate blood, soft tissues, respiratory tract, and urinary tract of an individual [2].

A. baumannii accounts for almost 90% of all reported Acinetobacter infections including ventilator-associated pneumonia, blood infection, soft tissue infection, peritonitis, urinary tract infections, and surgical site infections [3].

Multidrug resistance in *A.baumannii* is not a new phenomenon, *A.baumannii* is known to be intrinsically resistant to various antibiotics along with the ability to acquire genes that encode for resistance determinants [4].

Carbapenem and cephalosporins resistant *A. baumannii* are attrib¬uted to various reasons including produce Extended-spectrum beta-Lactamases (ESBL) and Metallo beta-lactamases (MBLs), reduced expression of outer membrane proteins and efficient efflux pumps [5, 6].

Production of beta-Lactamases, enzymes, diminished expression of outer membrane proteins, and up-regulation of efflux pumps play an important role in the mechanism of antibiotic resistance. Newer beta-Lactamases causing antimicrobial resistance include Extended-spectrum beta-Lactamases (ESBL) and Metallo-beta-Lactamases (MBL) [7].

The large scale use of third-generation cephalosporins like Cefotaxime, Ceftriaxone, and Ceftazidime has led to the evolution of newer beta-lactamases such as the ESBLs. ESBLs are plasmid-mediated enzymes that hydrolyze the lactam antibiotics and the carbapenems (Imipenem and Meropenem) [8].

Although the polymerase chain reaction technique is reliable and highly accurate, the instrument setting is costly and its accessibility is limited to the reference laboratories. Developing countries affected more on this issue. Another commercially available method, E-test which considers imipenem in one side of strip and Imipenem-EDTA on the other side is costly and insensitive in detecting most carbapenem susceptible MBL-harboring organisms, especially with *A. baumannii* isolates with MIC strip (≤ 4) [9].

This study aimed to determine the prevalence of ESBL and MBL production among strains of *A.baumannii* obtained from various clinical samples and antibiotic regimens. Resistance to the last-resort antibiotic treatment, Carbapenems, has reported more and more.

MATERIALS AND METHODS:

This is a cross-sectional study, a total of 55 *Acinetobacter baumannii* isolates from various clinical samples and total 6 month study from November 2019 to April 2020.

The anti-microbial susceptibility testing was performed using antibiotics obtained from Hi-Media, Mumbai, by the Kirby Bauer disk diffusion method, as per the guidelines of the Clinical Laboratory Standards Institute (CLSI) [10]. The samples comprised of Endotracheal aspirate, Blood, Pus, Sputum, Urine, and Swabs, *A.baumannii* strains isolated were identified by standard biochemical reactions.

Susceptibility to the following antibiotics (disc concentration) was tested Meropenem (10 µg), Imipenem (10 µg), Gentamycin (10µg); Ceftazidime (30µg); Piperacillin/Tazobactam (100+10 µg): Colistin (10µg), Polymyxin-B (300 units), Tobramycin (10 µg), Cefotaxime

(30 µg), Norfloxacin (10 µg)[10].

Phenotypic screening and confirmatory Detection of ESBL: Positive isolates resistant to 3rd generation cephalosporins (Ceftazidime, Cefotaxime, and Ceftriaxone) were tested for ESBL production by disc potentiation test. A disc of Ceftazidime (30 g) and Clavulanic acid or Amoxicillin (20 g /10 g) was placed 20mm apart, centre to centre on Mueller Hinton agar plate, and was incubated overnight at 37°C.

A zone difference greater than or equal to 5mm around Ceftazidime and Ceftazidime + Amoxiclave was interpreted as ESBL positive isolates [11].

Phenotypic detection of MBLs: To screen MBL producers in *Acinetobacter baumannii,* Imipenem and Meropenem resistant isolates were considered for phenotypic confirmatory detection.

Confirmatory Detection of MBL: The test organism adjusted to 0.5 McFarland's opacity standards was inoculated on Muller-Hinton agar plate. Two 10µg Imipenem discs one containing 750 µg EDTA, obtained from Hi-media, Mumbai were placed on the inoculated plate and incubated for 24hrs at 37°C. The single placed of Imipenem disc and zones of inhibition around this disc and Imipenem-EDTA disc was recorded. An increase in the zone size of inhibition diameter of at least 7mm around the Imipenem-EDTA disc as compared to Imipenem along with considered as a positive result [12].

RESULTS:

A total of 55 *Acinetobacter baumannii* isolates from all clinical samples. Out of total isolates, detection of Extended Spectrum Beta-Lactamases (ESBL) through screening method were resistance of 3rd generation cephalosporin's antibiotic, 45 (81.82%) Ceftazidime (30μg), Cefotaxime (30μg) and Ceftriaxone(30µg))and confirmatory detection by DDST method Ceftazidime (30μg) and Ceftazidime + Clavulanic acid (30μg/10μg) were 42 (76.36%) and MBL (Metallo-β-Lactamases) detection by the screening method Carbapenems resistance were 40 (72.73%)andconfirmatory detection EDTA DST method 35 (63.64%) respectively shown in (table no.1).

Table No.1:- Distribution of total *Acinetobacter baumannii* with MBL and ESBL Detection.

S. N.	Total *Acinetobacter baumannii*	ESBL		MBL	
		Screening Method	Confirmatory by DDST	Screening Method	Confirmatory by EDTA DST
1	**55 (100%)**	**45 (81.82%)**	**42 (76.36%)**	**40 (72.73%)**	**35 (63.64%)**

The total number of 55 isolates was *Acinetobacter baumannii*; the distribution of ESBL and MBL according to Sample-wise. Out of total isolates, most of the samples were Endotracheal Tube 35 (63.64%) & Blood 9 (16.36%). Detection of ESBL by phenotypic method out of 45 (81.82%) samples resistance of 3rd generation Cephalosporins were Endotracheal aspirate 31 (56.36%) & blood 6 (10.91%) and 42 (76.37%) ESBL confirmatory detection by DDST method were 30 (54.54%) Endotracheal aspirate & 5 (9.09%) Blood respectively. Out of 40 (72.73%) MBL Positive by Carbapenems resistance detection with Phenotypic Screening test Endotracheal aspirate 31 (56.36%) & Blood 5 (9.09%) and out of 35 (63.64%) Phenotypic Confirmatory by EDTA DST is Endotracheal Tube 30 (54.54%) & Blood 4 (7.27%) respectively shown in (Table No 2).

S.N.	SAMPLES	TOTAL	ESBL		MBL	
			Phenotypic Screening Method	**Phenotypic Confirmatory by DDST**	**Phenotypic Screening Method**	**Phenotypic Confirmatory by EDTADST**
1	Endotrachealaspirate	35(63.64%)	31(56.36%)	30(54.54%)	31(56.36%)	30(54.54%)
2	Blood	9(16.36%)	6(10.91%)	5(9.09%)	5(9.09%)	4(7.27%)
3	Sputum	4(7.27%)	2(3.64%)	2(3.64%)	1(1.82%)	0
4	Pus	3(5.45%)	3(5.45%)	2(3.64%)	2(3.64%)	1(1.82%)
5	Swab	2(3.63%)	1(1.82%)	1(1.82%)	0	0
6	Urine	2(3.60%)	2(3.64%)	2(3.64%)	1(1.82%)	0
7	**Total**	**55(100%)**	**45(81.82%)**	**42(76.37%)**	**40(72.73%)**	**35(63.64%)**

Table No. 2:- Distribution of samples with ESBL and MBL production in *Acinetobacter baumannii,* by phenotypic screening method and confirmatory method.

Acinetobacter baumannii isolates were 100% sensitive to Polymyxin B and Colistin, confirmed MBL & ESBL production. Resistance to 100% Ceftazidime and Ceftriaxone for ESBL & MBL producer Acinetobacter baumannii. Other sensitive to Meropenem and Imipenem was 29 (69.05%) & 26 (61.90%) confirmed ESBL whereas both are antibiotics 100% resistance in MBL producer shown in (table no.3).

S.N.	**ANTIBIOTICS**	**ESBL 42 (76.36%)**		**MBL 35 (63.64%)**	
		Sensitivity	**Resistance**	**Sensitivity**	**Resistance**
1	Polymyxin-B	42 (100%)	0	35 (100%)	0
2	Colistin	42 (100%)	0	35 (100%)	0
3	Imipenem	26 (61.90%)	16 (38.10%)	0	35 (100%)
4	Meropenem	29 (69.05%)	13 (30.95%)	0	35 (100%)
6	Norfloxacin	20 (47.62%)	22 (52.38%)	9 (25.71%)	26 (74.295)
7	Gentamicin	15 (35.71%)	27 (64.29%)	0	35 (100%)
8	Tobramycin	23 (54.76%)	19 (45.24%)	0	35 (100%)
9	Piperacillin/ Tazobactam	28 (66.67%)	14 (33.33%)	10 (28.57%)	25 (71.43%)
10	Ceftazidime	0	42 (100%)	0	35 (100%)
11	Cefotaxime	0	42 (100%)	0	35(100%)

Table No. 3:- Susceptibility pattern of *Acinetobacter baumannii* in confirmed ESBL and MBL production by DDST & EDTA DST method.

DISCUSSION:

Acinetobacter baumannii is an effective human microorganism in healthcare settings. A combination of its environmental flexibility and presence of multiple resistance determinants makes it a successful nosocomial pathogen.

In the present study out of 55 *Acinetobacter baumannii* were isolated in all clinical samples, a total of isolate Extended Spectrum Beta-Lactamases (ESBL) detection through confirmatory detection by DDST method were 42 (76.36%) and confirmatory detection MBL by EDTA DST method 35 (63.64%) respectively. Our study is compared to another study with near about 69 (63.3%) of the total Acinetobacter spp. isolated were ESBL producers Amrit Koirala1 *et al* [13] and MBL producer similar study found in Richa Hans *et al*. [14] where observed only 68% MBL production in *Acinetobacter baumannii*, and another findings were closely related to Kabbaj *et al*.[15] have reported MBL production in *A.baumannii* as 74%. However, in Brazil's study, most of the resistance of Carbapenemases was found to be 71.4% to 100% in various hospital settings in the Brazil region. [16].

In our result total number of 55 isolates was *Acinetobacter baumannii*, most of the sample was 42 (76.37%) ESBL confirmatory detection by DDST method, 30 (54.54%) Endotracheal aspirate & 5

(9.09%) Blood and out of 35 Phenotypic Confirmatory MBL detection by EDTA DST is most of the sample were Endotracheal aspirate 30 (54.54%) & Blood 4 (7.27%) respectively. Our result is compared with other results Jaggi *et.al* [17] and Muthusamy D *et.al.* [18] Where are results were 56% from and 18% from blood in ESBL and 73% from Endotracheal tips and, 23.8% from blood in MBL producer respectively.

In our study, *Acinetobacter baumannii* isolates were 100% sensitive to Polymyxin B and Colistin for, confirmed MBL & ESBL production. Resistance to 100% Ceftazidime and Ceftriaxone for ESBL & MBL producer *Acinetobacter baumannii*. Other sensitive to Meropenem and Imipenem was 29 (69.05%) & 26 (61.90%) confirmed ESBL whereas both are antibiotics 100% resistance in MBL producer. Our result is compared with Amandeep Kaur *et.al*.[19] which result was 100% sensitive to Polymyxin B & Colistin and 100% resistance to Ceftazidime & Ceftriaxone in ESBL detection and 77% to 100% resistance to Meropenem and Imipenem and other near about similar findings Marzieh Safari et. al. [20], which result was 98%, 98% and 99% out of 100 *A. baumannii* isolates were resistant to Meropenem , Imipenem, Ceftazidime and Cefotaxime, respectively.

A. baumannii is recommended to perform these simple phenotypic tests like EDTA DST Test for MBL and DDST test for ESBL production in microbiology laboratories to determine susceptibility patterns and prevent in prejudice use of antimicrobial agents.

CONCLUSION:

The current study shows that ESBL and MBL production in *Acinetobacter baumannii* is on the rise across the world, thus making these infections difficult to treat. Polymyxin B and Colistin were 100% sensitive against *Acinetobacter baumannii* Early phenotypic detection of ESBL and MBL production would be important for the reduction of mortality rate and the spread of multidrug-resistant organisms. Moreover, it is important to implement antibiotic policies to avoid excessive and injudicious use of extended-spectrum cephalosporins and carbapenems in every hospital for the best treatment.

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